REMARKS

I. Status of the Claims

Claims 1-5 and 9-25 are pending in the application. Claims 1, 3-5, 9-21 and 23-25 stand rejected, variously, under 35 U.S.C. §112, first and second paragraphs, and 35 U.S.C. §102. The specific grounds for rejection, and applicants' response thereto, are set out in detail below.

New claims 36-43 are provided and are supported by the original claims.

II. Rejection Under 35 U.S.C. §112, First Paragraph

Claims 1, 3-5, 9-21 and 23-25 stand rejected under the first paragraph of §112 as lacking enablement. According to the examiner, while the claims are enabled for protecting a mouse from an organophosphate comprising administering to the mouse an expression construct comprising a CMV promoter linked to a PON1 gene, the specification does not provide enablement using promoters generally. Applicants traverse.

The examiner's only evidentiary support for the argument that not all promoters will work derives from Furlong *et al.*, which support is misplaced. Furlong can be distinguished in two ways from the present claims. First, Furlong was in fact seeking to make transgenic animals. That is ***not*** applicants' intention here, as exemplified by the fact that their examples show a ***non-integrative*** virus (adenovirus). Thus, to suggest that the promoter selection for the present claims must support long-term stable expression, as Furlong was attempting, simply is incorrect. Second, Furlong's paper was published in 1994, some 7 years prior to the instant application's priority date. And third, Furlong's statements regarding the lack of understanding regarding PON1 regulatory mechanisms and cis-acting elements is similarly irrelevant since

applicants' choice of heterologous promoters avoids such issues, which would be associated with the ***native*** PON1 promoter.

Thus, it is respectfully submitted that the examiner has not made out a *prima facie* case of lack of enablement for promoters generally. Reconsideration and withdrawal of the rejection is therefore respectfully requested.

III. Rejection Under 35 U.S.C. §112, Second Paragraph

Claims 1-5 and 9-25 stand rejected as allegedly indefinite in failing to recite all necessary steps. It is argued that the step of exposing the cell or subject to an organophosphate is required. Applicants traverse. Because the organophosphate is a toxin, there is no intentional "exposing" – this may or may not happen as a result of the cell or subject entering an environment where the organophosphate exists or appears. Those persons providing the PON1 gene would not be the same persons (if any) introducing the organophosphate. Thus, what the examiner suggests for the claim to be "complete" is, in fact, illogical. That said, applicants have amended the wherein clauses of claims 1 and 21 so that they track more closely with and fully satisfy the claim preamble. Reconsideration and withdrawal of the rejection is therefore respectfully requested.

IV. Rejections Under 35 U.S.C. §102 and §103

Claims 1-5, 91-5 and 17-25 are rejected as anticipated by, and claims 1 and 19 are rendered obvious by (in conjunction with Mackness *et al.*), Hudson *et al.* (U.S. Patent 5,629,193). Applicants traverse.

According to the examiner, Hudson *et al.* teaches the administration of a PON1 gene using a variety of vectors, for the purpose of preventing cell death due to organophosphate toxicity. However, the examiner is incorrect in citing Hudson *et al.* as teaching PON1. Hudson

et al. teaches ***PON2***, which explains why they advanced and secured composition of matter claims – indeed, had they been attempting to claim PON1 sequences, they would have failed because PON1 was previously described in the literature (see Hassett *et al.*, "Characterization of cDNA clones encoding rabbit and human serum paraoxonase: the mature protein retains its signal sequence," *Biochemistry* 30(42):10141-9, Oct 22, 1991; attached). What Hudson *et al.* failed to appreciate at the time of filing, likely because the had obtained ***absolutely no data whatsoever***, was that they had cloned an enzyme that operated quite differently than PON1. In any event, Hudson *et al.* cannot anticipate the claims of the present application because the sequence provided in SEQ ID NOS:1 and 2 and in FIG. 1A-B is ***not*** PON1, but PON2 (see attached sequence information).

Turning to the obviousness rejection, applicants would direct the examiner to statements made in the first office action, mailed on August 23, 2005, where it was stated that as of 2001-2002, gene therapy was "unpredictable." If gene therapy was unpredictable in 2001-2002, then it was nearly ***unattainable*** as of 1994, when Hudson *et al.* was filed. In this regard, the complete and utter absence of ***any*** data in Hudson *et al.* regarding the ability to transfer nucleic acids into living organisms and provide protection is a glaring deficiency that cannot be overlooked. To suggest that the scant disclosure provided by Hudson *et al.* renders obvious applicants' invention, which is supported by *in vivo* data, contradicts the examiner's stance on the record regarding gene therapy. Furthermore, reliance on Mackness might provide a correct citation to PON1, but it fails to establish that one could achieve, via gene therapy, protection of subjects from organophosphate toxins. As such, it is submitted that the obviousness rejection cannot stand.

V. Conclusion

In light of the foregoing, applicants submit that all claims are in condition for allowance, and an early notification to that effect is earnestly solicited. Should the examiner have any questions regarding this response, a telephone call to the undersigned is invited.

Please date stamp and return the enclosed postcard as evidence of receipt.

Respectfully submitted,

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Characterization of cDNA Clones Encoding Rabbit and Human Serum Paraoxonase: The Mature Protein Retains Its Signal Sequence[†,‡]

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ABSTRACT: Serum paraoxonase hydrolyzes the toxic metabolites of a variety of organophosphorus insecticides. High serum paraoxonase levels appear to protect against the neurotoxic effects of organophosphorus substrates of this enzyme [Costa et al. (1990) *Toxicol. Appl. Pharmacol. 103*, 66–76]. The amino acid sequence accounting for 42% of rabbit paraoxonase was determined by (1) gas-phase sequencing of the intact protein and (2) peptide fragments from lysine and arginine digests. From these data, two oligonucleotide probes were synthesized and used to screen a rabbit liver cDNA library. A clone was isolated and sequenced, and contained a 1294-bp insert encoding an open reading frame of 359 amino acids. Northern blot hybridization with RNA isolated from various rabbit tissues indicated that paraoxonase mRNA is synthesized predominately, if not exclusively, in the liver. Southern blot experiments suggested that rabbit paraoxonase is coded by a single gene and is not a family member of closely related genes. Human paraoxonase clones were isolated from a liver cDNA library by using the rabbit cDNA as a hybridization probe. Inserts from three of the longest clones were sequenced, and one full-length clone contained an open reading frame encoding 355 amino acids, four less than the rabbit paraoxonase protein. Each of the human clones appeared to be polyadenylated at a different site, consistent with the absence of the canonical polyadenylation signal sequence. Of potential significance with respect to the paraoxonase polymorphism, the derived amino acid sequence from one of the partial human cDNA clones differed at two positions from the full-length clone. Amino-terminal sequences derived from purified rabbit and human paraoxonase proteins suggested that the signal sequence is retained, with the exception of the initiator methionine residue [Furlong et al. (1991) *Biochemistry* (preceding paper in this issue)]. Characterization of the rabbit and human paraoxonase cDNA clones confirms that the signal sequences are not processed, except for the N-terminal methionine residue. The rabbit and human cDNA clones demonstrate striking nucleotide and deduced amino acid similarities (greater than 85%), suggesting an important metabolic role and constraints on the evolution of this protein.

Polymorphic genes encoding human biotransformation enzymes which result in variable rates of metabolism of certain drugs and xenobiotics have been identified. Examples of polymorphic enzymes include cytochrome P450 isozymes which hydroxylate the antihypertensive drug debrisoquine and the anticonvulsant mephenytoin (Kalow, 1987), an *N*-acetyltransferase which metabolizes arylamine and hydrazine compounds (Weber, 1987), the glutathione transferase μ isozyme which conjugates glutathione to electrophilic compounds (Seidegard et al., 1988), and serum cholinesterase which metabolizes the anesthetic succinylcholine (Brown et al., 1981).

Paraoxonase, like serum cholinesterase, demonstrates a substrate-dependent polymorphism in human populations [see Geldmacher-von Malinckrodt and Diepgen (1988) for review]. Some paraoxonase substrates, such as phenylacetate and chlorpyrifos oxon, are hydrolyzed with the same turnover number by both allelic forms of the enzyme, whereas paraoxon is hydrolyzed slowly by one allelic form and rapidly by the other (LaDu et al., 1986; Furlong et al., 1989, Smolen et al., 1991). It has been suggested that high serum levels of paraoxonase may be protective against poisoning by organophosphate substrates of this enzyme (Omenn, 1987; LaDu & Eckerson, 1984; Furlong et al., 1988, 1989). Experiments with animal systems support this hypothesis (Main, 1956; Costa et al., 1990).

One of our aims is to determine the molecular basis for the paraoxonase polymorphism observed in humans. Because rabbits have very high levels of paraoxonase (Costa et al., 1987), we first purified and partially sequenced rabbit paraoxonase (Furlong et al., 1991). The protein sequence data were used to design oligonucleotide probes which permitted the isolation of a rabbit cDNA. The rabbit clone was subsequently used as a probe to isolate human paraoxonase cDNAs. This report describes these cloning experiments and presents the structural characterization of rabbit and human paraoxonase.

Materials and Methods

Protein Purification. Paraoxonase was purified through the DEAE-cellulose fractionation step as described previously (Furlong et al., 1991). Paraoxonase was further purified by

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[‡] The nucleotide sequences in this paper have been submitted to the GenBank/EMBL Data Bank under Accession Numbers M63011, M63012, M63013, and M63014.

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high-performance chromatography on a 5-μm Vydac C_{18} column.

Protein Digests and Peptide Purification. Paraoxonase was pyridylethylated and succinylated as described by Crabb et al. (1988). Pyridylethylated HPLC-purified paraoxonase was fragmented at lysyl residues with endoproteinase Lys-C (Crabb et al., 1986). Pyridylethylated, succinylated DEAE-purified paraoxonase was cleaved at arginyl residues with trypsin (Crabb et al., 1986). Peptides were purified by narrow-bore reverse-phase HPLC using an Applied Biosystems Model 130 HPLC system.

Protein/Peptide Sequencing. Intact paraoxonase and fractionated peptides were sequenced with an Applied Biosystems gas-phase sequencer (Model 470) and an on-line phenylthiohydantoin amino acid analyzer (Model 120) using the 03RPTH sequencer program and the manufacturer's recommended program and solvents for the PTH analyzer (Crabb et al., 1988). Phenylthiocarbamyl (PTC) amino acid analysis was performed according to West and Crabb (1990) using an Applied Biosystems automatic system (Models 420H/130/920).

Oligonucleotide Synthesis. DNA probes and primers were synthesized with an Applied Biosystems DNA synthesizer using phosphoramidite chemistry.

Library Screening and Subcloning. (A) Rabbit. A λgt11 cDNA library constructed from the pooled livers of male and female New Zealand white rabbits was obtained from Clontech (Palo Alto, CA). The library was screened as described previously (Hassett & Omiecinski, 1987; Hassett et al., 1989) by using the oligonucleotide probes described under Results. The rabbit insert was subcloned into pUC13 with *Escherichia coli* DH5α as host (BRL, Gaithersburg, MD).

(B) Human. A λgt11 human liver cDNA library derived from an adult female was also obtained from Clontech. This library was screened with the 952-bp *Bst*XI restriction fragment from rabbit paraoxonase cDNA. Inserts were subcloned in pSK(+) Bluescript plasmid vector and used to transform XL1-Blue cells (Stratagene, La Jolla, CA).

DNA Sequence Analysis. (A) Rabbit. The insert cDNA was sequenced directly in pUC13 by using the forward and reverse universal plasmid primers and the 17-base paraoxonase-specific primer. Insert DNA was subcloned into the vector in both orientations, relative to the multiple cloning site. Unique *Bam*HI and *Hin*dIII restriction sites in the insert DNA and in the vector cloning region allowed deletion constructs to be engineered which facilitated sequence analysis of both strands from the universal primers. Each DNA strand was sequenced at least three times.

(B) Human. The nucleotide sequence of the human DNA clones was determined in the plasmid by using primers complementary to the T3 and T7 promoters of the vector. Additionally, 11 oligonucleotide primers were synthesized for sequencing on the basis of the derived human and rabbit sequences.

DNA was sequenced by using the dideoxy termination method (Sanger et al., 1977) and Sequenase Version 2.0 (U.S. Biochemicals, Cleveland, OH), as described previously (Hassett & Omiecinski, 1990). Sequence analysis and database searches were performed with either GENEPRO (Riverside Scientific Enterprises, Bainbridge Island, WA) or Intelligenetics (Palo Alto, CA) software and databases, which included GenBank and EMBL DNA databases and the PIR protein database.

Northern Blot Analysis. RNA was isolated (Omiecinski et al., 1985) from the liver, lung, kidney, and testes of two New Zealand White rabbits. Twenty micrograms of total RNA from each organ was size-fractionated in a 6% formaldehyde/1.15% agarose gel and transferred to a GeneScreen Plus nylon membrane as per the manufacturer's directions (Du Pont/NEN, Boston, MA). A 438-bp *Bam*HI fragment isolated from the 3′ region of the rabbit paraoxonase cDNA was radiolabeled (Hassett & Omiecinski, 1990) and used as a hybridization probe. The membrane was washed at 45 °C in 0.1× SSC/0.1% SDS (1× SSC: 1.5 M NaCl, 0.15 M sodium citrate) and exposed overnight to X-ray film in the presence of two intensifying screens. The size of the in vivo RNA transcript was estimated by using an RNA ladder standard (BRL, Gaithersburg, MD).

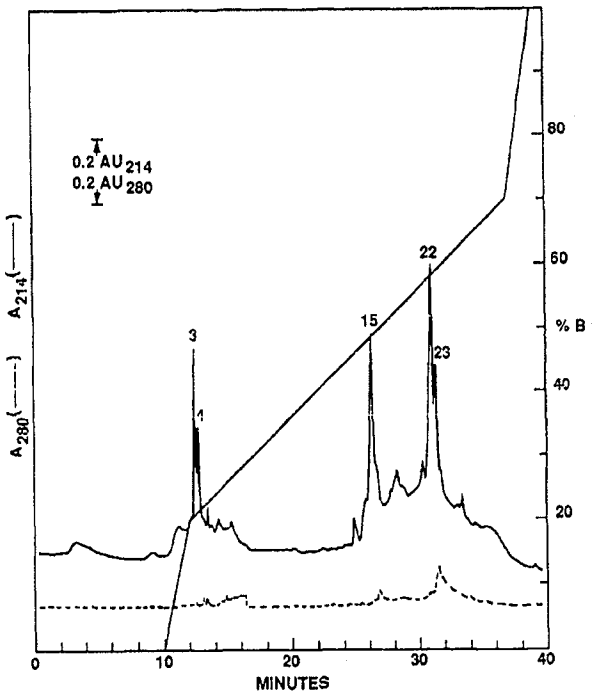

FIGURE 1: Reverse-phase HPLC purification of rabbit paraoxonase. Paraoxonase was purified through the DEAE-Trisacryl M step (87 μg) and fractionated by reverse-phase HPLC on a 5-μm Vydac C18 column. Solvent A was 0.1% trifluoroacetic acid in H_2O, and solvent B was 84% acetonitrile containing 0.09% trifluoroacetic acid.

Southern Blot Analysis. Peripheral white blood cell DNA was extracted and isolated from 5 mL of whole blood withdrawn from a single rabbit and processed essentially as described (Blin & Stafford, 1976). Twenty micrograms of DNA was digested with *Eco*RI, *Bam*HI, *Hin*dIII, *Pst*I, or *Xho*I, size-fractionated on a 0.85% agarose gel, and transferred to a nylon membrane as described previously (Hassett et al., 1989). The Southern blot was incubated with a radiolabeled 419-bp fragment isolated from the rabbit paraoxonase cDNA (*Eco*RI/*Bam*HI fragment). The blot was washed in a final solution of 0.1× SSC/0.1% SDS at 50 °C and exposed to X-ray film for 6 days in the presence of two intensifying screens. Drigest III (Pharmacia, Piscataway, NJ) was employed as a molecular size standard.

RESULTS

Purification of Rabbit Paraoxonase. Rabbit paraoxonase, purified through the DEAE-Trisacryl M step as described in the preceding paper, was further purified by high-performance reverse-phase liquid chromatography (Figure 1). Peak 15 contained only homogeneous paraoxonase while peaks 22 and 23 contained both paraoxonase and apolipoprotein A1 (de-

Table I: Rabbit Paraoxonase Protein/Peptide Sequence[a]

protein/peptide	sequence	position
amino terminus	AKLTALTLLGLGLALFDGQKS-FQT	2-26
lysyl peptides	S-FQTRFNVHREVTPVELPN-NL	22-44
	LIPSVNDIVAVGPEHFYA	163-180
	IHVYEK	245-250
	SLDFNTLVDNISVDPV	261-276
	NPPASEVLRIQDIL	298-311
	ALY-ELSQAN	350-359
arginyl peptides	FNVHR	28-32
	VVAEGFDFANGINISPDGKYVYIAELLAHKI-VY	215-248
	IFYYDPKNPPASEVLR	291-306
	IQDILSKEPKV-VAYAE	307-323

[a] Cycles where no residue was assigned are shown as dashes. Tentative assignments are underlined. The single assignment which differed from the deduced sequence shown in Figure 3 is italicized. Sequences that were used for probe design are shown in bold type.

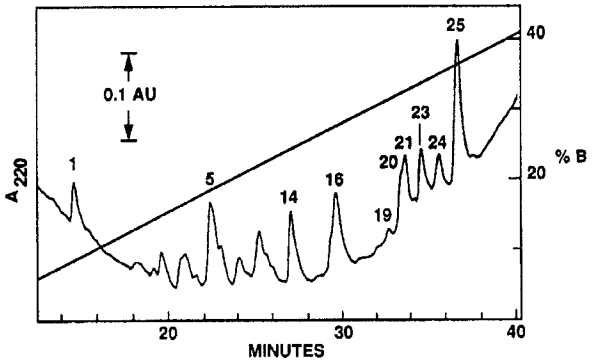

FIGURE 2: HPLC purification of lysine peptides from paraoxonase. Peptides resulting from the cleavage of pyridylethylated paraoxonase (~78 μg) with endopeptidase Lys-C were purified by narrow-bore reverse-phase HPLC with a linear gradient of buffer A (0.1% TFA) to 50% buffer B (85% acetonitrile, 0.005% TFA) run over 40 min.

termined by sequence analysis).

Peptide Generation. RP-HPLC-purified pyridylethylated paraoxonase was digested with endoproteinase Lys-C, and the resulting peptides were purified by narrow-bore RP-HPLC (Figure 2) as described under Materials and Methods. In addition, paraoxonase (200 μg) purified by DEAE-Trisacryl M chromatography (preceding paper) was succinylated and then subjected to arginyl-specific cleavage with trypsin, and the resulting peptides were purified by narrow-bore RP-HPLC. Since the intact paraoxonase had not been RP-HPLC-purified prior to digestion, peptides from apolipoprotein A1 were also identified by sequence analysis (data not shown).

Gas-Phase Protein Sequencing. Unequivocal amino acid sequencing data were obtained from rabbit paraoxonase, four arginyl peptides, and six lysyl peptides (Table I). Residues 16-20 of the amino terminus of rabbit paraoxonase (Phe-AspGlyGlnLys) allowed the design of the 15-base oligonucleotide 5'-TTY GAY GGN CAR AAR-3'[1] with 64-fold redundancy (Table I, Figure 3). A 17-base oligomer (5'-GGR TCR TAR TAR AAD AT-3') with 48-fold redundancy was designed as a complement to the nucleotide sequence encoding residues 1-6 of the arginine peptide IFYYDP (Table I, Figure 3). The wobble position of the proline codon was not used in the design of this oligomer.

Isolation and Sequence of Rabbit Paraoxonase cDNA. Approximately 400000 plaques were screened from the rabbit cDNA library with the 15-base probe, yielding 35 potentially positive autoradiographic signals. Twenty-four of these phage were rescreened with the 17-base probe, and a plaque which hybridized to this oligomer was plated at a low density. This cDNA clone was screened a final time with the 15-base probe and once again showed positive hybridization. Phage DNA was purified, digested with *Eco*RI, and subcloned into pUC13.

DNA sequence analysis of the rabbit paraoxonase cDNA (RabPON, GenBank Assession Number M63011) identified an insert of 1294 bp, containing the entire protein coding sequence (Figure 3). Fifty-one nucleotides precede the methionine initiation codon, ATG, which begins an open reading frame coding for 359 amino acids. An amber stop codon, TAG, is followed by an additional 163 nucleotides of 3' noncoding sequence. The ATG at position 1 is the likely start position since there is a stop codon beginning 15 nucleotides upstream from this ATG. No poly(A) signal or sequence was identified in this clone. The fragment containing this information was presumably deleted during library construction since multiple efforts to isolate this region from the original λ phage were unsuccessful.

Comparison between the Derived and Determined Amino Acid Sequences. The deduced amino acid sequence is shown in Figure 3. The sequence verified by gas-phase amino acid sequencing is presented in Table I. The verified protein sequence totaled 151 residues or about 42% of the rabbit paraoxonase protein sequence deduced from the cDNA clone. The one difference observed (i.e., Ile for Leu at position 164) may simply reflect a variant in the rabbit population.

Analysis of Paraoxonase mRNA Expression in Rabbit Tissues. Northern blot analysis performed with RNA isolated from four rabbit organs revealed the presence of paraoxonase-specific RNA in liver only. RNA isolated from lung, kidney, or testes did not hybridize to the paraoxonase cDNA probe (Figure 4). On the basis of this Northern blot and linear regression analysis, the molecular size estimate for the in vivo liver mRNA transcript was approximately 1400 bases. Pretreatment of animals with phenobarbital 16 h prior to sacrifice did not influence steady-state mRNA levels of liver paraoxonase (data not shown).

Evaluation of Rabbit Paraoxonase Gene Complexity. Southern-blotted rabbit genomic DNA was digested with five restriction endonucleases prior to electrophoresis and probed with the 400-bp *Eco*RI/*Bam*HI fragment of the rabbit paraoxonase cDNA. In each restriction digest lane, only one hybridization band was observed (Figure 5). These data suggest that rabbit paraoxonase protein is probably encoded by a single gene, and not a member of a family of closely related genes.

Isolation and Sequence of Human Paraoxonase cDNAs. Approximately 300000 plaques from the human cDNA library were screened with a 952-bp *Bst*XI radiolabeled fragment from the rabbit paraoxonase cDNA. From this library screen, 41 plaques were identified, and the three longest clones were

[1] As recommended by the Nomenclature Committee of the International Union of Biochemistry, nucleotides are abbreviated as follows: R = purine; Y = pyrimidine; N = A, T, G, or C; D = G, A, or T.

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RabPON                                                  CGG CCC   -46

    AGC CCG TGG TGC TCG CGC CGG TCC AGC CTT TAG TCT GCC CTG ACC    -1
HuPON1                                              CCC CCG ACC    -1

    ATG GCT AAA CTG ACA GCG CTC ACG CTC TTG GGG CTG GGA TTG GCA    45
    Met Ala Lys Leu Thr Ala Leu Thr Leu Leu Gly Leu Gly Leu Ala
    Met Ala Lys Leu Ile Ala Leu Thr Leu Leu Gly Met Gly Leu Ala        15
    ATG GCG AAG CTG ATT GCG CTC ACC CTC TTG GGG ATG GGA CTG GCA

    CTC TTC GAT GGA CAG AAG TCT TCT TTC CAA ACA CGA TTT AAT GTT    90
    Leu Phe Asp Gly Gln Lys Ser Ser Phe Gln Thr Arg Phe Asn Val
    Leu Phe Arg Asn His Gln Ser Ser Tyr Gln Thr Arg Leu Asn Ala        30
    CTC TTC AGG AAC CAC CAG TCT TCT TAC CAA ACA CGA CTT AAT GCT

    CAC CGT GAA GTA ACT CCA GTG GAA CTT CCT AAC TGT AAT TTA GTT   135
    His Arg Glu Val Thr Pro Val Glu Leu Pro Asn Cys Asn Leu Val
    Leu Arg Glu Val Gln Pro Val Glu Leu Pro Asn Cys Asn Leu Val         45
    CTC CGA GAG GTA CAA CCC GTA GAA CTT CCT AAC TGT AAT TTA GTT

    AAA GGG ATT GAC AAT GGT TCT GAA GAC TTG GAA ATA CTG CCC AAT   180
    Lys Gly Ile Asp Asn Gly Ser Glu Asp Leu Glu Ile Leu Pro Asn
    Lys Gly Ile Glu Thr Gly Ser Glu Asp Met Glu Ile Leu Pro Asn         60
    AAA GGA ATC GAA ACT GGC TCT GAA GAC ATG GAG ATA CTG CCT AAT

    GGA CTG GCT TTC ATC AGC GCC GGA TTA AAA TAT CCT GGA ATA ATG   225
    Gly Leu Ala Phe Ile Ser Ala Gly Leu Lys Tyr Pro Gly Ile Met
    Gly Leu Ala Phe Ile Ser Ser Gly Leu Lys Tyr Pro Gly Ile Lys         75
    GGA CTG GCT TTC ATT AGC TCT GGA TTA AAG TAT CCT GGA ATA AAG

    AGC TTT GAC CCT GAT AAG CCT GGA AAG ATA CTT CTA ATG GAC CTG   270
    Ser Phe Asp Pro Asp Lys Pro Gly Lys Ile Leu Leu Met Asp Leu
    Ser Phe Asn Pro Asn Ser Pro Gly Lys Ile Leu Leu Met Asp Leu         90
    AGC TTC AAC CCC AAC AGT CCT GGA AAA ATA CTT CTG ATG GAC CTG

    AAT GAG AAA GAC CCA GTA GTA TTG GAA CTG AGC ATT ACT GGA AGT   315
    Asn Glu Lys Asp Pro Val Val Leu Glu Leu Ser Ile Thr Gly Ser
    Asn Glu Glu Asp Pro Thr Val Leu Glu Leu Gly Ile Thr Gly Ser        105
    AAT GAA GAA GAT CCA ACA GTG TTG GAA TTG GGG ATC ACT GGA AGT

    ACA TTT GAT TTA TCT TCA TTT AAC CCT CAT GGG ATT AGC ACA TTC   360
    Thr Phe Asp Leu Ser Ser Phe Asn Pro His Gly Ile Ser Thr Phe
    Lys Phe Asp Val Ser Ser Phe Asn Pro His Gly Ile Ser Thr Phe        120
    AAA TTT GAT GTA TCT TCA TTT AAC CCT CAT GGG ATT AGC ACA TTC

    ACA GAT GAA GAT AAT ATC GTC TAC CTG ATG GTG GTG AAC CAT CCA   405
    Thr Asp Glu Asp Asn Ile Val Tyr Leu Met Val Val Asn His Pro
    Thr Asp Glu Asp Asn Ala Met Tyr Leu Leu Val Val Asn His Pro        135
    ACA GAT GAA GAT AAT GCC ATG TAC CTC CTG GTG GTG AAC CAT CCA

    GAT TCC AAG TCC ACA GTG GAG TTG TTT AAA TTC CAA GAA AAA GAA   450
    Asp Ser Lys Ser Thr Val Glu Leu Phe Lys Phe Gln Glu Lys Glu
    Asp Ala Lys Ser Thr Val Glu Leu Phe Lys Phe Gln Glu Glu Glu        150
    GAT GCC AAG TCC ACA GTG GAG TTG TTT AAA TTT CAA GAA GAA GAA

    AAA TCA CTT TTG CAT CTG AAA ACC ATC AGA CAC AAG CTT CTG CCT   495
    Lys Ser Leu Leu His Leu Lys Thr Ile Arg His Lys Leu Leu Pro
    Lys Ser Leu Leu His Leu Lys Thr Ile Arg His Lys Leu Leu Pro        165
    AAA TCG CTT TTG CAT CTA AAA ACC ATC AGA CAT AAA CTT CTG CCT

    AGT GTG AAT GAC ATT GTC GCT GTG GGA CCT GAA CAC TTT TAT GCT   540
    Ser Val Asn Asp Ile Val Ala Val Gly Pro Glu His Phe Tyr Ala
    Asn Leu Asn Asp Ile Val Ala Val Gly Pro Glu His Phe Tyr Gly        180
    AAT TTG AAT GAT ATT GTT GCT GTG GGA CCT GAG CAC TTT TAT GGC

    ACC AAT GAT CAC TAT TTT ATT GAC CCT TAC TTA AAA TCC TGG GAA   585
    Thr Asn Asp His Tyr Phe Ile Asp Pro Tyr Leu Lys Ser Trp Glu
    Thr Asn Asp His Tyr Phe Leu Asp Pro Tyr Leu Gln Ser Trp Glu        195
    ACA AAT GAT CAC TAT TTT CTT GAC CCC TAC TTA CAA TCC TGG GAG

    ATG CAT TTG GGA TTA GCG TGG TCA TTT GTT ACT TAT TAT AGT CCC   630
    Met His Leu Gly Leu Ala Trp Ser Phe Val Thr Tyr Tyr Ser Pro
    Met Tyr Leu Gly Leu Ala Trp Ser Tyr Val Val Tyr Tyr Ser Pro        210
    ATG TAT TTG GGT TTA GCG TGG TCG TAT GTT GTC TAC TAT AGT CCA

    AAT GAT GTT CGA GTA GTG GCA GAA GGA TTT GAT TTT GCT AAC GGA   675
    Asn Asp Val Arg Val Val Ala Glu Gly Phe Asp Phe Ala Asn Gly
    Ser Glu Val Arg Val Val Ala Glu Gly Phe Asp Phe Ala Asn Gly        225
    AGT GAA GTT CGA GTG GTG GCA GAA GGA TTT GAT TTT GCT AAT GGA

    ATC AAC ATC TCA CCA GAC GGC AAG TAT GTC TAT ATA GCT GAA CTG   720
    Ile Asn Ile Ser Pro Asp Gly Lys Tyr Val Tyr Ile Ala Glu Leu
    Ile Asn Ile Ser Pro Asp Gly Lys Tyr Val Tyr Ile Ala Glu Leu        240
    ATC AAC ATT TCA CCC GAT GGC AAG TAT GTC TAT ATA GCT GAG TTG
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CTG GCT CAT AAG ATC CAT GTG TAT GAA AAG CAC GCT AAT TGG ACT   765
Leu Ala His Lys Ile His Val Tyr Glu Lys His Ala Asn Trp Thr
Leu Ala His Lys Ile His Val Tyr Glu Lys His Ala Asn Trp Thr        255
CTG GCT CAT AAG ATT CAT GTG TAT GAA AAG CAT GCT AAT TGG ACT

TTA ACT CCA TTG AAG TCC CTC GAC TTT AAC ACT CTT GTG GAC AAC   810
Leu Thr Pro Leu Lys Ser Leu Asp Phe Asn Thr Leu Val Asp Asn
Leu Thr Pro Leu Lys Ser Leu Asp Phe Asn Thr Leu Val Asp Asn        270
TTA ACT CCA TTG AAG TCC CTT GAC TTT AAT ACC CTC GTG GAT AAC

ATA TCC GTG GAT CCT GTG ACA GGG GAC CTT TGG GTT GGT TGT CAT   855
Ile Ser Val Asp Pro Val Thr Gly Asp Leu Trp Val Gly Cys His
Ile Ser Val Asp Pro Glu Thr Gly Asp Leu Trp Val Gly Cys His        285
ATA TCT GTG GAT CCT GAG ACA GGA GAC CTT TGG GTT GGA TGC CAT

CCC AAT GGC ATG CGA ATC TTC TAC TAT GAC CCA AAG AAT CCT CCT   900
Pro Asn Gly Met Arg Ile Phe Tyr Tyr Asp Pro Lys Asn Pro Pro
Pro Asn Gly Met Lys Ile Phe Phe Tyr Asp Ser Glu Asn Pro Pro        300
CCC AAT GGC ATG AAA ATC TTC TTC TAT GAC TCA GAG AAT CCT CCT

GCA TCA GAG GTG CTT CGA ATC CAG GAC ATT TTA TCC AAA GAG CCC   945
Ala Ser Glu Val Leu Arg Ile Gln Asp Ile Leu Ser Lys Glu Pro
Ala Ser Glu Val Leu Arg Ile Gln Asn Ile Leu Thr Glu Glu Pro        315
GCA TCA GAG GTG CTT CGA ATC CAG AAC ATT CTA ACA GAA GAA CCT

AAA GTG ACA GTG GCT TAT GCA GAA AAT GGC ACT GTG TTA CAG GGC   990
Lys Val Thr Val Ala Tyr Ala Glu Asn Gly Thr Val Leu Gln Gly
Lys Val Thr Gln Val Tyr Ala Glu Asn Gly Thr Val Leu Gln Gly        330
AAA GTG ACA CAG GTT TAT GCA GAA AAT GGC ACA GTG TTG CAA GGC

AGC ACG GTG GCC GCT GTG TAC AAA GGG AAA ATG CTG GTT GGC ACC  1035
Ser Thr Val Ala Ala Val Tyr Lys Gly Lys Met Leu Val Gly Thr
Ser Thr Val Ala Ser Val Tyr Lys Gly Lys Leu Leu Ile Gly Thr        345
AGT ACA GTT GCC TCT GTG TAC AAA GGG AAA CTG CTG ATT GGC ACA

GTG TTC CAC AAA GCT CTC TAC TGT GAG CTC TCA CAG GCC AAT TAG  1080
Val Phe His Lys Ala Leu Tyr Cys Glu Leu Ser Gln Ala Asn *** 359
Val Phe His Lys Ala Leu Tyr Cys Glu Leu ***                 355
GTG TTT CAC AAA GCT CTT TAC TGT GAG CTC TAA CAG ACC GAT TTG

CAC CCG TGC CGC GGA CAC TGG CAC CCA CGA TTT CAA CTG CTT GCC  1125
CAC CCA TGC CAT AGA AAC TGA GGC CAT TAT TTC AAC CGC TTG CCA

GGC CAC ATT CTT GGG GCC ACA GTG CCC TCG GCG GGA TGA TGG ACA  1170
TAT TCC GAG GAC CCA GTG TTC TTA GCT GAA CAA TGA ATG CTG ACC

ACC CTA AAT TTG ACA TCA ACT GCA TCG CAG CCT AGA GTG GAT ATG  1215
CTA AAT GTG GAC ATC ATG AAG CAT CAA AGC ACT GTT TAA CTG GGA

AAG AGT AGG GCT TTT TGA GCG TGA ATT C                       1243
GTG ATA TGA TGT GTA GGG CTT TTT TTT GAG AAT ACA CTA TCA AAT  1260

CAG TCT TGG AAT ACT TGA AAA CCT CAT TTA CCA TAA AAA TCC TTC  1305

TCA CTA AAA TGG ATA AAT CAG TTA AAA AAA AA                   1337
```

FIGURE 3: Nucleotide and deduced amino acid sequences of RabPON and HuPON1 cDNAs. The rabbit sequences are presented in normal font in the upper lines; the human sequences are italicized in the lower lines. Alignment begins at the initiation codon ATG, which is arbitrarily designated position 1. Nucleotides preceding this codon are assigned negative numbers. The regions used for oligomer construction in the rabbit sequence are identified by an overline. Amino acid differences between RabPON and HuPON1 are boxed. Potential N-glycosylation sites are shown in bold type.

characterized by DNA sequencing (GenBank Assession Numbers: HuPON1, M63012; HuPON2, M63013; HuPON3, M63014). The DNA sequence of the these clones indicated that only HuPON1 was full length (Figure 3). This 1337-bp cDNA, including a 9-base poly(A) tail at the 3′ end, contained an open reading frame of 1065 bases that predicted a 355 amino acid protein. Clones HuPON2 and HuPON3 have 5′ termini starting 62 and 96 nucleotides downstream from the 5′ end of clone HuPON1, respectively. The nucleotide sequences of clones HuPON1 and HuPON2 predict a methionine at position 55 and glutamine at position 192, while clone HuPON3 predicts a protein with a leucine (TTG) at position 55 and an arginine (CGA) at position 192. The former substitution results in the loss of a restriction site (*Nla*III) in HuPON3, while the latter substitution creates *Alw*I and *Sau*3A sites in HuPON3.

Comparison of Rabbit and Human Paraoxonase Sequences. Alignment of rabbit and human cDNA coding regions revealed an 86% identity (Figure 3). The protein sequences deduced from these clones indicated an 85% identity, which increases to 88.7% when conservative amino acid substitutions are considered (Figure 3). The deduced rabbit amino acid sequence contains five potential N-glycosylation sites, whereas the human sequence predicts four possible N-glycosylation sites.

Discussion

The most difficult step in isolating a cDNA clone for human serum paraoxonase has been obtaining sufficient pure enzyme from which to obtain a protein sequence that in turn could be used to design oligomer probes for library screening. We overcame this problem by purifying and partially sequencing paraoxonase from rabbits, which have much higher levels of paraoxonase than humans and for which an activity stain was developed (preceding paper). These sequence data were used to design oligonucleotide probes which enabled the isolation of a rabbit paraoxonase cDNA. The rabbit clone was used to isolate corresponding human liver cDNA clones.

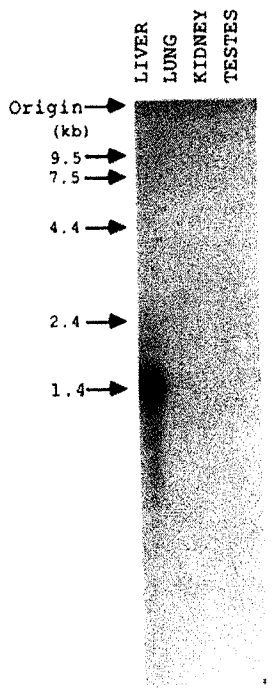

FIGURE 4: Northern blot analysis of rabbit RNA. Total RNA was isolated from the liver, lung, kidney, and testes of untreated rabbits and size-separated in an agarose/formaldehyde gel. Following tranfer to a nylon membrane, the blot was probed with the RabPON cDNA. Molecular size standards are shown in the left margin. A single hybridization band is observed only in liver, suggesting an in vivo transcript of approximately 1.4 kb.

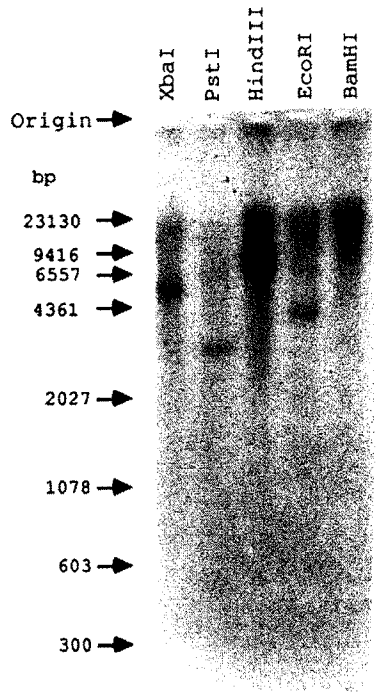

FIGURE 5: Southern blot analysis of rabbit genomic DNA. Twenty micrograms of DNA was digested under excess conditions with each of the indicated restriction enzymes, size-separated in an agarose gel, and transferred to a nylon membrane which was hybridized with a radioactive RabPON cDNA fragment. Molecular size markers are shown in the left margin.

The full-length rabbit and human clones demonstrate extensive conservation in nucleotide and deduced amino acid sequences despite the evolutionary distance separating these species. Although the predicted length of the two proteins differs by four amino acids, no gaps were required for the alignment of these sequences. A region of absolute conservation between the two sequences is observed from amino acids 213 to 275. Furthermore, within these 63 residues, three of the four predicted N-glycosylation sites common to the two proteins occur. Although the rabbit and human paraoxonase clones demonstrate significant conservation, other genes or proteins related to paraoxonase were not identified in database searches, despite the fact that many sequences have been described for proteins which perform similar catalytic functions (e.g., esterase activity). On the basis of the unreported partial peptide sequence from human paraoxonase, Gan et al. (1991) also did not identify closely related sequences in database searches.

Comparison of the deduced protein sequences from RabPON and HuPON1 cDNAs to the amino-terminal sequences determined by gas-phase sequencing of the intact proteins reveals a unique feature of paraoxonase. Both the rabbit and human enzymes retain their signal sequences, with only the amino-terminal methionine residues cleaved. Database searches indicate that the N-terminal sequences of rabbit and human paraoxonase show similarity to other protein secretion signal sequences (Figure 6). Conservation of specific amino acids is apparent, but particularly interesting are the conserved three amino acid residues LAL. An inspection of other published signal sequences (Watson, 1984) indicates that many of these also contain this sequence in the hydrophobic core region.

We are unaware of other examples where typical nonmutant signal sequences are retained in mature, secreted proteins. Cleavage of signal sequences appears to follow certain rules (von Heijne, 1983). Both the human and rabbit sequences possess a positively charged amino terminus commonly found in signal sequences, as well as a 9-residue hydrophobic core starting at position 9. In eukaryotes, cleavage typically occurs 5–6 residues from the C-terminal boundary of the hydrophobic core, which would predict a cleavage site for the paraoxonase proteins following residue 22 or 23. Furthermore, accurate processing is thought to exclude certain residues at the −1 and −3 positions, relative to the cleavage site (von Heijne, 1986). On the basis of the cleavage site positions predicted above, the −3 position would be occupied in the paraoxonase protein by Gln_{20} or Lys_{21} (rabbit) or by His_{20} or Gln_{21} (human). These are "forbidden" residues in the −3 position and may explain why the signal sequence of paraoxonase is retained.

The function of the retained signal sequence is unknown. The "hydrophobic head" of paraoxonase may be important for interaction with the high-density lipoprotein particle with which it is intimately associated. Detergents are required to dissociate paraoxonase from apolipoprotein A1 (Furlong et al., preceding paper; Gan et al., 1991). Hydrophobicity analyses (Figure 7) clearly show the hydrophobic amino termini of rabbit and human paraoxonases, as well as considerable hydrophobic character in the remainder of the proteins.

Comparison of the full-length HuPON1 cDNA with the two human partial clones reveals two interesting features. First, two nucleotide substitutions result in amino acid differences between clones HuPON1 and HuPON2 vs HuPON3. It is not known if either of these substitutions accounts for the differences observed between high- and low-activity paraoxonase allelic forms. In this regard, it is of interest to compare the two amino acid substitutions predicted from clone HuPON3 with the orthologous positions predicted from the

	MAKLTALTLLGLGLALFDGQKSSFQTR	Rabbit Paraoxonase
	MAKLIALTLLGMGLALFRNHQSSYQTR	Human Paraoxonase
	MQMSPALTCLVLGLALVFGEGSAVHHP	Plasminogen Activator inhibitor-1 precursor, Human[1]
	MAPRTLLLLLSGALALTQTWARSHSMR	HLA alpha chain precursor, clone pHLA$_1$ Human[2]
4	MAPRTLILLLSGALALTETWAGSHSMR	HLA alpha chain precursor, cw3, Human[3]
4	MAPRTLLLLLAGALTLKDTQAGSHSMR	RLA alpha chain precursor histocompatibility antigen, Rabbit[4]
	MAPCTLLLLLAAALAPTQYRAGPHSLR	H-2 k-d alpha chain precursor class 1 antigen[5]
	MAKLLALSLSFCFLLLGGCFALREQPQ	Legumin A precursor Garden pea[6]
	MGKKSHICCFSLLLLLFAGLASGHQVL	α amylase 2-precursor Barley[7]
	MAAATTTTSRPLLLSRQQAAASSLQCR	Fructose 1, 6 bisphosphatase precursor wheat[8]
3	TAPRTVLLLLSAALALTETWAGSHSMR	CHLA-81 α chain precursor histocompatibility antigen chimpanzee[9]
7	ASRLTLLTLLLLLLLAGDRASSNPNATS	Complement C1 inhibitor precursor - Human[10]
3	ATKTFALLLLSLFLAVGLGEKKEGHFS	Gastric inhibitor polypeptide precursor Human[11]
	MRMLLHLSLLALGAAYVYAIPTEIPTS	Interleukin 5 Human[12]
	MQMSPALTCLVLGLTLVFGEGSAVHHP	Plasminogen activator inhibitor-1 precursor Human[13]
7	MQKLLKCSRLVLALALILVLESSVQGY	Secretory granule proteoglycan core protein precursor-Human[14]
	MAGPPRLLLLPLLLALARGLPGALAAQ	T cell surface glycoprotein CD7 precursor Human[15]
7	EAPIVLLLLLWLALAPTPGSASSEAPP	Ribophorin I precursor Rat[16]

FIGURE 6: Comparison of the amino-terminal signal sequence regions of rabbit and human paraoxonases with similar signal sequences found in searching the DNA/protein databases. Numbers appearing to the left of the sequences indicate the residue position. Other sequences being at the first residue. Footnotes: (1) Pannekoek et al., 1986; Ginsburg et al., 1986; (2) Malissen et al., 1982; (3) Sodoyer et al., 1984; (4) Tykocinski et al., 1984; (5) Kvist et al., 1983; Lalanne et al., 1983; (6) Lycett et al., 1984; (7) Knox et al., 1987; (8) Raines et al., 1988; (9) Meyer et al., 1988; (10) Bock et al., 1986; (11) Takeda et al., 1987; (12) Azuma et al., 1986; (13) Strandberg et al., 1988; (14) Stevens et al., 1988; (15) Aruffo & Seed, 1987; (16) Harnik-Ort et al., 1987.

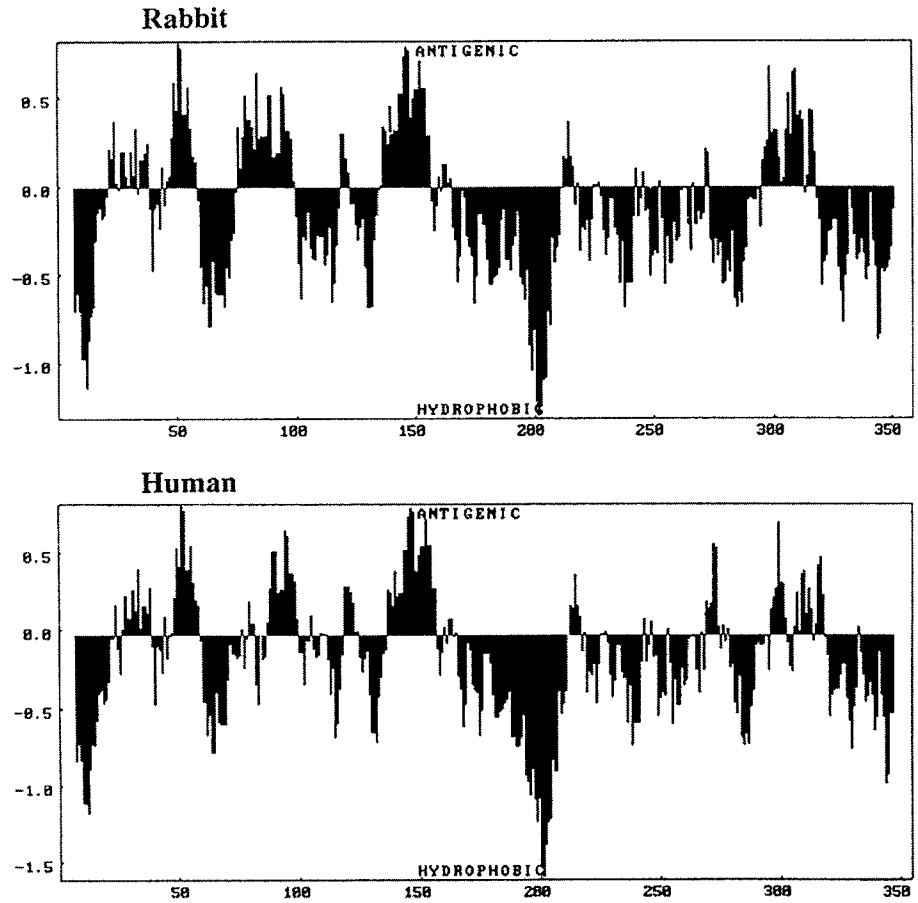

FIGURE 7: Hydrophobicity profiles of rabbit and human paraoxonase. The analysis was performed with GENEPRO software according to the Hopp and Woods (1981) algorithm with a window setting of 12.

rabbit cDNA. Amino acid 55 is a Leu in both sequences, whereas residue 192 is conservatively substituted (Lys in RabPON, Arg in HuPON3). It is tempting to speculate that since rabbits have high-activity paraoxonase, HuPON3 (which shares two similar and potentially important amino acids) might represent the high-activity allele genotype in the human. A gene frequency for the low-activity paraoxonase allele of 0.69 would predict a 43% probability that a given person would be a heterozygote (Furlong et al., 1989). Therefore, it is not unlikely that the individual from whom the human liver library was constructed was heterozygous for the paraoxonase allele, expressing mRNA for both high- and low-activity forms of the enzyme.

The importance of these amino acid changes could be examined by different approaches. Expressing the human cDNAs in vitro, or site-directed mutagenesis targeting the

```
HuPON1:  TAACAGACCGATTTGCACCCATGCCATAGAAACTGAGGCCATTATTTCAACCGCTTGCCA   60
HuPON2:  TAACAGACCGATTTGCACCCATGCCATAGAAACTGAGGCCATTATTTCAACCGCTTGCCA
HuPON3:  TAACAGACCGATTTGCACCCATGCCATAGAAACTGAGGCCATTATTTCAACCGCTTGCCA
         ************************************************************

HuPON1:  TATTCCGAGGACCCAGTGTTCTTAGCTGAACAATGAATGCTGACCCTAAATGTGGACATC  120
HuPON2:  TATTCCGAGGACCCAGTGTTCTTAGCTGAACAATGAATGCTGACCCTAAATGTGGACATC
HuPON3:  TATTCCGAGGACCCAGTGTTCTTAGCTGAACAATGAATGCTGACCCTAAATGTGGACATC
         ************************************************************

HuPON1:  ATGAAGCATCAAAGCACTGTTTAACTGGGAGTGATATGATGTGTAGGGCTTTTTTTTGAG  180
HuPON2:  ATGAAGCATCAAAGCACTGTTTAACTGGGAGTGATATGATGTGTAGGGCTTTTTTTTGAG
HuPON3:  ATGAAGCATCAAAGCACTGTTTAACTGGGAGTGATATGATGTGTAGGGCTTTTTTTTGAG
         ************************************************************

HuPON1:  AATACACTATCAAATCAGTCTTGGAATACTTGAAAACCTCATTTACCATAAAAATCCTTC  240
HuPON2:  AATACACTATCAAATCAGTCTTGGAAAAAAAAAAAAAA                       218
HuPON3:  AATACACTATCAAATCAGTCTTGGAATACTTGAAAACCTCATTTACCATAAAAATCCTTC
         ************************

HuPON1:  TCACTAAAATGGATAAATCAGTTAAAAAAAAA                             272
HuPON3:  TCACTAAAATGGATAAATCAGTTATGTCAATTGTCAGATATTAAATAACAGTGTGTGACC  300

HuPON3:  CCAAAAGTACTTACCCTAAAACATGTGTTGCCTGAAAGCACATGTGTGTATCGCTGCCTT  360

HuPON3:  GCCATGTCTTGTTCAGAAGACACAGGGGAGCAGGGTTAGGTCACGTGTCTTTAGAACTCC  420

HuPON3:  AGTACTCACCCAGGGACTCCAGTTCACAGGCCAGAAAACATATGCATTATGAAGTTCCCC  480

HuPON3:  TCTACTCCATGCACATAGTAAGTCTGACTATGGCAGTCAGACTTACTTACTCCCATTTTC  540

HuPON3:  CCTTCGATATATGACTTTTTCTCAGTAAATATTAACCTGAACTATTCCAAAAAAAAAAAA  600
HuPON3:  AAAAAAAAA                                                    609
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FIGURE 8: Comparison of the 3′ noncoding portions of the human paraoxonase cDNAs. HuPON1, HuPON2, and HuPON3 are aligned and numbered beginning with the termination codon TAA, shown in bold type. Identical nucleotide residues in all sequences are indicated with an asterisk below the aligned residue. Potential polyadenylation signal sequences are underlined.

nucleotides encoding these amino acids, could reveal a concordant relationship with substrate-dependent metabolism and the human polymorphism. A more general approach for the identification of genetic alterations relevant to the paraoxonase polymorphism would be to sequence genomic DNA isolated from individuals characterized for high and low activity and to search for structural differences common to each group. Restriction site differences observed between the human sequences should also be useful in this regard.

A second observation in comparing the human clones is the different lengths of the 3′ untranslated regions, shown in Figure 8. The sequences are consistent with the existence of mRNAs which are polyadenylated at different sites. The canonical polyadenylation signal (AATAAA) is not found in any of these clones, although potential alternative poly(A) signal sequences are present. The probable polyadenylation signals CATAAA or ACTAAA (HuPON1), AATACA (HuPON2), and AGTAAA (HuPON3) are thought to be polyadenylated and cleaved inefficiently (Sheets et al., 1990). It may be relevant that Gieselman et al. (1989) found that individuals with arylsulfatase A pseudodeficiency had a point mutation of the polyadenylation signal of the arylsulfatase A gene, which resulted in a substantial reduction in the amount of normal message. The amount of arylsulfatase protein and arylsulfatase enzyme activity was reduced 90% in individuals with arylsulfatase A pseudodeficiency. A second mutation affecting a glycosylation site was present in individuals with arylsulfatase A pseudodeficiency, but was found not to affect enzyme activity. Variation up to 13-fold in paraoxonase/arylesterase enzyme activity between individuals with the same allozyme type (e.g., homozygous low paraoxonase activity) has been observed (Furlong et al., 1989), and the levels observed are stable over time. It remains to be determined whether variations in polyadenylation signals between individuals of a given allozyme type contribute to the observed stable differences in enzyme levels. Alterations in the 5′ regulatory region and stable differences in transcription factor levels could also contribute to or be responsible for these differences.

The physiological substrate for paraoxonase has not been identified.

ACKNOWLEDGMENTS

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GenBank Entry for Pon1

```
LOCUS       NM_000446               2395 bp    mRNA    linear   PRI 21-JAN-2007
DEFINITION  Homo sapiens paraoxonase 1 (PON1), mRNA.
ACCESSION   NM_000446
VERSION     NM_000446.3  GI:31880793
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 2395)
  AUTHORS   Mastorikou,M., Mackness,M. and Mackness,B.
  TITLE     Defective metabolism of oxidized phospholipid by HDL from people
            with type 2 diabetes
  JOURNAL   Diabetes 55 (11), 3099-3103 (2006)
   PUBMED   17065348
  REMARK    GeneRIF: In the control group, there was a significant negative
            correlation between serum PON1 activity and oxidized LDL
            concentration, but not in people with type 2 diabetes.
REFERENCE   2  (bases 1 to 2395)
  AUTHORS   Gaidukov,L., Rosenblat,M., Aviram,M. and Tawfik,D.S.
  TITLE     The 192R/Q polymorphs of serum paraoxonase PON1 differ in HDL
            binding, lipolactonase stimulation, and cholesterol efflux
  JOURNAL   J. Lipid Res. 47 (11), 2492-2502 (2006)
   PUBMED   16914770
  REMARK    GeneRIF: PON1 192R/Q polymorphs differ in HDL binding,
            lipolactonase stimulation, and cholesterol efflux
REFERENCE   3  (bases 1 to 2395)
  AUTHORS   Hofer,S.E., Bennetts,B., Chan,A.K., Holloway,B., Karschimkus,C.,
            Jenkins,A.J., Silink,M. and Donaghue,K.C.
  TITLE     Association between PON 1 polymorphisms, PON activity and diabetes
            complications
  JOURNAL   J. Diabetes Complicat. 20 (5), 322-328 (2006)
   PUBMED   16949520
  REMARK    GeneRIF: Genotyping of adolescents with diabetes for PON 1
            polymorphisms was performed, including that of a novel PON 1
            promoter polymorphism A(-1074)G. PON genotypes were related to
            diabetes complication status.
REFERENCE   4  (bases 1 to 2395)
  AUTHORS   Lakshman,M.R., Gottipati,C.S., Narasimhan,S.J., Munoz,J.,
            Marmillot,P. and Nylen,E.S.
  TITLE     Inverse correlation of serum paraoxonase and homocysteine
            thiolactonase activities and antioxidant capacity of high-density
            lipoprotein with the severity of cardiovascular disease in persons
            with type 2 diabetes mellitus
  JOURNAL   Metab. Clin. Exp. 55 (9), 1201-1206 (2006)
   PUBMED   16919539
  REMARK    GeneRIF: cross-sectional study to correlate PON-1, homocysteine
            thiolactonase activities, and the lag time of LDL oxidation in
            control subjects and subjects with type 2 diabetes mellitus with
            different degrees of CVD
REFERENCE   5  (bases 1 to 2395)
  AUTHORS   Yamane,T., Matsumoto,T., Nakae,I., Takashima,H., Tarutani,Y.,
            Tamaki,S. and Horie,M.
  TITLE     Impact of paraoxonase polymorphism (Q192R) on endothelial function
            in intact coronary circulation
  JOURNAL   Hypertens. Res. 29 (6), 417-422 (2006)
   PUBMED   16940704
  REMARK    GeneRIF: as estimated by bradykinin and acetylcholine testing,
            findings suggest that paraoxonase-1 (PON1) genotypes may not play a
            critical role in the modulation of endothelial vasomotor function
            in the intact coronary circulation.
REFERENCE   6  (bases 1 to 2395)
  AUTHORS   Adkins,S., Gan,K.N., Mody,M. and La Du,B.N.
  TITLE     Molecular basis for the polymorphic forms of human serum
            paraoxonase/arylesterase: glutamine or arginine at position 191,
            for the respective A or B allozymes
  JOURNAL   Am. J. Hum. Genet. 52 (3), 598-608 (1993)
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PUBMED 7916578
REFERENCE 7 (bases 1 to 2395)
AUTHORS Humbert,R., Adler,D.A., Disteche,C.M., Hassett,C., Omiecinski,C.J. and Furlong,C.E.
TITLE The molecular basis of the human serum paraoxonase activity polymorphism
JOURNAL Nat. Genet. 3 (1), 73-76 (1993)
PUBMED 8098250
REFERENCE 8 (bases 1 to 2395)
AUTHORS Hassett,C., Richter,R.J., Humbert,R., Chapline,C., Crabb,J.W., Omiecinski,C.J. and Furlong,C.E.
TITLE Characterization of cDNA clones encoding rabbit and human serum paraoxonase: the mature protein retains its signal sequence
JOURNAL Biochemistry 30 (42), 10141-10149 (1991)
PUBMED 1657140
REFERENCE 9 (bases 1 to 2395)
AUTHORS Gan,K.N., Smolen,A., Eckerson,H.W. and La Du,B.N.
TITLE Purification of human serum paraoxonase/arylesterase. Evidence for one esterase catalyzing both activities
JOURNAL Drug Metab. Dispos. 19 (1), 100-106 (1991)
PUBMED 1673382
REFERENCE 10 (bases 1 to 2395)
AUTHORS Ortigoza-Ferado,J., Richter,R.J., Hornung,S.K., Motulsky,A.G. and Furlong,C.E.
TITLE Paraoxon hydrolysis in human serum mediated by a genetically variable arylesterase and albumin
JOURNAL Am. J. Hum. Genet. 36 (2), 295-305 (1984)
PUBMED 6324579
COMMENT VALIDATED REFSEQ: This record has undergone preliminary review of the sequence, but has not yet been subject to final review. The reference sequence was derived from CB122285.1 and Z70723.1.
On Jun 18, 2003 this sequence version replaced gi:19923105.

Publication Note: This RefSeq record includes a subset of the publications that are available for this gene. Please see the Entrez Gene record to access additional publications.

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compare to sequence in Fig.1A and 1B. Doesn't match.

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GenBank entry for Pon2 (the coding sequence in Patent US 5,629,193)

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VERSION NM_000305.2 GI:66529293
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Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini; Catarrhini; Hominidae; Homo.

REFERENCE 1 (bases 1 to 1669)
AUTHORS Ng,C.J., Bourquard,N., Grijalva,V., Hama,S., Shih,D.M., Navab,M., Fogelman,A.M., Lusis,A.J., Young,S. and Reddy,S.T.
TITLE Paraoxonase-2 deficiency aggravates atherosclerosis in mice despite lower apolipoprotein-B-containing lipoproteins: anti-atherogenic role for paraoxonase-2
JOURNAL J. Biol. Chem. 281 (40), 29491-29500 (2006)
PUBMED 16891303
REMARK GeneRIF: Plays a protective role against atherosclerosis in vivo.

REFERENCE 2 (bases 1 to 1669)
AUTHORS Slowik,A., Tomik,B., Wolkow,P.P., Partyka,D., Turaj,W., Malecki,M.T., Pera,J., Dziedzic,T., Szczudlik,A. and Figlewicz,D.A.
TITLE Paraoxonase gene polymorphisms and sporadic ALS
JOURNAL Neurology 67 (5), 766-770 (2006)
PUBMED 16822965
REMARK GeneRIF: the C311S polymorphism was associated with sALS in dominant and additive models.

REFERENCE 3 (bases 1 to 1669)
AUTHORS Saeed,M., Siddique,N., Hung,W.Y., Usacheva,E., Liu,E., Sufit,R.L., Heller,S.L., Haines,J.L., Pericak-Vance,M. and Siddique,T.
TITLE Paraoxonase cluster polymorphisms are associated with sporadic ALS
JOURNAL Neurology 67 (5), 771-776 (2006)
PUBMED 16822964
REMARK GeneRIF: A haploblock of high linkage disequilibrium (LD) spanning PON2 and PON3 was associated with SALS.

REFERENCE 4 (bases 1 to 1669)
AUTHORS Draganov,D.I., Teiber,J.F., Speelman,A., Osawa,Y., Sunahara,R. and La Du,B.N.
TITLE Human paraoxonases (PON1, PON2, and PON3) are lactonases with overlapping and distinct substrate specificities
JOURNAL J. Lipid Res. 46 (6), 1239-1247 (2005)
PUBMED 15772423
REMARK GeneRIF: PON1, PON2, and PON3 are lactonases with overlapping and distinct substrate specificities

REFERENCE 5 (bases 1 to 1669)
AUTHORS Ng,C.J., Shih,D.M., Hama,S.Y., Villa,N., Navab,M. and Reddy,S.T.
TITLE The paraoxonase gene family and atherosclerosis
JOURNAL Free Radic. Biol. Med. 38 (2), 153-163 (2005)
PUBMED 15607899
REMARK Review article

REFERENCE 6 (bases 1 to 1669)
AUTHORS Ng,C.J., Wadleigh,D.J., Gangopadhyay,A., Hama,S., Grijalva,V.R., Navab,M., Fogelman,A.M. and Reddy,S.T.
TITLE Paraoxonase-2 is a ubiquitously expressed protein with antioxidant properties and is capable of preventing cell-mediated oxidative modification of low density lipoprotein
JOURNAL J. Biol. Chem. 276 (48), 44444-44449 (2001)
PUBMED 11579088

REFERENCE 7 (bases 1 to 1669)
AUTHORS Hong,S.H., Song,J., Min,W.K. and Kim,J.Q.
TITLE Genetic variations of the paraoxonase gene in patients with coronary artery disease
JOURNAL Clin. Biochem. 34 (6), 475-481 (2001)
PUBMED 11676977
REMARK GeneRIF: study suggested a gene-gene interaction between the PON1 and PON2 polymorphisms for CAD risk; may have linkage disequilibrium with a tightly linked PON3 locus or significant atherosclerotic alleles of nearby genes

REFERENCE 8 (bases 1 to 1669)
AUTHORS Mochizuki,H., Scherer,S.W., Xi,T., Nickle,D.C., Majer,M., Huizenga,J.J., Tsui,L.C. and Prochazka,M.
TITLE Human PON2 gene at 7q21.3: cloning, multiple mRNA forms, and missense polymorphisms in the coding sequence
JOURNAL Gene 213 (1-2), 149-157 (1998)
PUBMED 9714608
REFERENCE 9 (bases 1 to 1669)
AUTHORS Sanghera,D.K., Aston,C.E., Saha,N. and Kamboh,M.I.
TITLE DNA polymorphisms in two paraoxonase genes (PON1 and PON2) are associated with the risk of coronary heart disease
JOURNAL Am. J. Hum. Genet. 62 (1), 36-44 (1998)
PUBMED 9443862
REFERENCE 10 (bases 1 to 1669)
AUTHORS Primo-Parmo,S.L., Sorenson,R.C., Teiber,J. and La Du,B.N.
TITLE The human serum paraoxonase/arylesterase gene (PON1) is one member of a multigene family
JOURNAL Genomics 33 (3), 498-507 (1996)
PUBMED 8661009
COMMENT REVIEWED REFSEQ: This record has been curated by NCBI staff. The reference sequence was derived from CB961097.1, AF001601.1 and BC040010.1.
On May 24, 2005 this sequence version replaced gi:4505952.

Summary: This gene encodes a member of the paraoxonase gene family, which includes three known members located adjacent to each other on the long arm of chromosome 7. The encoded protein is ubiquitously expressed in human tissues, membrane-bound, and may act as a cellular antioxidant, protecting cells from oxidative stress. Hydrolytic activity against acylhomoserine lactones, important bacterial quorum-sensing mediators, suggests the encoded protein may also play a role in defense responses to pathogenic bacteria. Mutations in this gene may be associated with vascular disease and a number of quantitative phenotypes related to diabetes. Alternatively spliced transcript variants encoding different isoforms have been described.

Transcript Variant: This variant (1) represents the longer transcript and encodes the longer isoform (1).

Publication Note: This RefSeq record includes a subset of the publications that are available for this gene. Please see the Entrez Gene record to access additional publications.
COMPLETENESS: complete on the 3' end.

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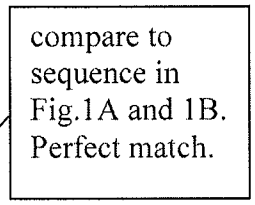


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